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Assessment of phenotypic developmental traits and hybrid vigour in *Arabidopsis thaliana*

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The Don-0 and Col-0 ecotypes of *Arabidopsis thaliana* showed significant phenotypic diversity for several quantitative traits. The phenotypic data taken on hybrid derived from Col-0 x Don-0 and parental lines provides the information of their continuous developmental pattern throughout the life cycle. The developmental data exhibits heterosis for most of the traits (specifically biomass related traits) due to supplementary genetic contributions of both parents. Consequently, the hybrid was found to be more vigorous in comparison to both the parents in terms of all the traits except yield trait. Moreover, higher and lower performance of hybrid causes due to constructive (hybrid vigor) and deleterious epistatic interactions of loci (necrosis: hybrid incompatibility), respectively. The present study also concluded that Don-0 is a slow growing, delayed flowering and high biomass containing ecotype. The shoot and root biomass at initial stage was found to be less in Don-0 than the Col-0 but high at maturity. The present investigation provides the preliminary information which could be utilized for future genetic studies to understand the heterotic phenomenon in *Arabidospsis*.

Keywords: *Arabidopsis thaliana*, phenotypic traits, developmental stages, hybrid, heterosis, SNP

Introduction

The *Arabidopsis* belongs to the family Brassicaceae and exists with numerous species i.e. *A. thaliana*, *A. suecica*, *A. arenosa*, *A. neglecta*, *A. croatica*, *A. cebennensis*, *A. pedemontana*, *A. lyrata* and *A. halleri*[1]. The *A. thaliana* has been extensively utilized for several studies related to molecular biology, genetics, developmental biology, physiology, stress biology and breeding due to its short life cycle and resource availability. In nature, the variation plays important role in plant system for their adaptation and survival under adverse conditions[2]. However, the plants develop variations at phenotypic level (leaf structure, trichome, shoot and root biomass) and genotypic level (expression and regulation of gene, methylation and silencing events) by altering the metabolic processes[3]. The strains of *A. thaliana* originated from same ancestor and further evaluated through internal transcribed spacers of nuclear ribosomal DNA[4]. At global level, good number of wild genotypes or accessions of *A. thaliana* (approximately 6000) have been composed from diverse ecological locations[5]. Although, morphological traits have been deliberated to explore genetic diversity associated with stress using wild type Columbia (Col-0) and mutants plant in *Arabidopsis*[6]. Identification of novel genes/alleles would be possible through exploration of diversity which may be associated with, higher plant performance under different environmental conditions[7].

Genetic diversity of natural accessions has been exploited to dissect the complex genetic interactions and molecular markers have been used to investigate the diversity at genetic level in plants. The various markers such as simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), cleaved amplified polymorphic sequences (CAPS), simple sequence length polymorphism (SSLP)[8-12] and single nucleotide polymorphism (SNP) markers[13-14] has also been utilized to explore the molecular diversity in *A. thaliana.* Among various markers, the SNP is considered as most advance and has been widely explored in several plants like barley[15], oil palm[16], rapeseed[17], *Jatropha*[18] etc. The hybridization between two diverse genotypes may produce superior hybrid for particular

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agronomical/economical traits. Moreover, heterosis is generated through interactions between the two parental genomes and/or epigenomes in the nucleus of the hybrid which may lead higher (hybrid necorsis) vigour[19] or lower (hybrid necrosis)[20] performance due to interaction of favourable or deleterious genes/alleles respectively. The hybrid vigour has been explained by dominance and over-dominance mechanism which alters gene expression, molecular pathways. In *A. thalliana* some heterosis related studies has been carried out using different ecotypes such as Col-0, C24, Nd, Ws-2, Wei-0, Aa-0, Ler, Alt-5 and Bod-6[20-23]. The correlation between heterosis and gene expression have been established for cellular and molecular processes using Col-0 × C24 as parental lines[24]. However, heterosis is considered as a complex phenomenon and various studies tried to explain it at phenotypic and genetic level[25-26]. In the present investigation, we tried to understand the pattern of phenotypic variability of important traits among hybrid derived from Col-0 x Don-0. This cross combination of Col-0 x Don-0 is utilized for the first time to investigate heterotic performance related to various morphological traits in *A. thaliana*.

Materials and Methods

Plant Materials

Two ecotypes of *A. thaliana* i.e. Col-0 and Don-0 were selected as parents for the development of hybrid. The Col-0 and Don-0 were selected on the basis of variations found at ecological, molecular[27-28] and at morphological level (studied at CSIR-National Botanical Research Institute (NBRI), Lucknow, India). Morphological variations were observed for several phenotypic parameters related to flowering time, root and shoot biomass traits etc. Seeds of these two ecotypes (Don-0 and Col-0) were obtained from *Arabidopsis* Biological Resource Centre (ABRC), Ohio State University (https://abrc.osu.edu/) and are being maintained at CSIR-NBRI, Lucknow, India.

Glasshouse Conditions for Growth of *A. thaliana* Plants

All the experiments were performed under the glasshouse conditions. The Col-0, Don-0 and hybrids were grown at 22°C in a commercial soil mix containing soilrite (Keltech Energies Ltd., Bengaluru, India) and vermiculite (3:1) and on Murashige and Skoog (MS) agar medium[29] under the following growth conditions: a 16 hr light/8 hr dark photoperiod, 250 µmol m^{-2} s^{-1} light intensity and 80% relative humidity. Seeds were sown in soilrite such that one ended up with 10-20 plants per pot. Further, pots were placed in trays with optimum amount of Osgrel Somerwhile solution media (OS media) followed by covering with plastic wrap and stratification (4°C for 3 day). Then plants were transferred to glass house and allowed to grow. Depending upon requirement, the plants were given OS media with alternate day intervals as their nutrient supply. One plant per pot was allowed to grow for phenotypic evaluation.

Development of Hybrid

The hybridization between Col-0 (female) and Don-0 (male) has been performed manually with help of hand held magnifying glass. The unopened flower buds were firstly tagged with fine thread and then emasculated with the help of fine forcep and bagged to avoid cross pollination. Next day, the pollen from Don-0 was dusted on the stigma of emasculated flowers of Col-0 and allowed to form fruit and seeds. The mature fruits (siliques) were harvested and hybrid seeds/ F_1 seeds collected.

DNA Isolation and Hybrid Confirmation

The total genomic DNA was isolated from fresh leaves of parents and hybrid using DNAzol method (Invitrogen, USA) following manufacturer's protocol. The quality and quantity of DNA was checked on 0.8% agarose gel and fluorometer (Qubit, Invitrogen) respectively. The genomic DNA was diluted to 10 ng/µl for PCR amplification. The hybridity was confirmed using SSR markers selected from Cosson *et al*[30]. The amplified PCR product was checked on 3% agarose gel and documented on Bio-rad gel documentation system.

Phenotyping of Col-0, Don-0 and F_1 Hybrid

The phenotypic data was recorded on Col-0, Don-0 and hybrids (total 6 plants for each individual) grown in soil media for developmental traits. The data were measured at every seventh day under glasshouse conditions. The traits included were 1) *Bolting days*: calculated from date of sowing to emergence of unopened bud, 2) *Days to flowering*: the number of days after sowing to at which the first flower opened, 3) *Rosette diameter*: measured with scale (cm) which covered maximum circular area of rosette leaves, 4) *Rosette leaf length*: measured manually from initiation to tip of leaf with scale (cm) in triplicates (includes 3 mature rosette leaves per plants), 5) *Rosette leaf width:* measured in middle leaf section manually using scale (cm) in triplicates, 6) *Number of*

rosette leaves: counted within the whole rosette structure, 7) *Number of cauline leaves*: counted after bolting when visible nodes and leaves appeared in stem, 8) *Cauline leaf length*: measured after flowering on first, second and third node of the plant from base to top of plant, 9) *Cauline leaf width*: measured after flowering on first, second and third node of plant, 10) *Trichome density*: counted under microscope with 1X coverage in 3 blocks of 0.5 cm^2 area per leaf of individual plants (9 blocks of 0.5 cm^2 area/3 leaves of particular plant), 11) *Number of internodes*: counted after flowering when visible nodes and cauline leaves appeared on stem, 12) *Internode distance*: measured (cm) as space between the two nodes, 13) *Plant height:* measured (cm) at maturity, 14) *Root length*: plant was isolated from the soil at their maturity and measured the root length using scale (cm) and 15) *Root dry biomass*: dried the isolated root of plant at room temperature and measured root dry biomass using weighing balance (mg). The developmental data of two traits i.e. root length and root dry biomass was analyzed in one month gap through isolation of plant root from soil media. In addition, 16) *Number of secondary roots*, 17) *Length of secondary root* and (18) *Number of axillary root* were also measured on MS media with one day interval. Further, siliques length, siliques weight and seed number were also calculated at maturity.

Result

In the present study, two ecotypes of *A. thaliana* have been selected based on previous studies carried out at CSIR-NBRI, Lucknow. The ecotype Col-0 showed early bolting and flowering as compared to Don-0 (Fig. 1A & B). The number of rosette leave, cauline leaves and their length, width has also showed distinct variations between Col-0 and Don-0 ecotypes. In general, the Col-0 has one leaf at their most of the nodes which is known as paraclade (Fig. 1C). In contrast, cauline leaves of Don-0 created aerial rosette like structure on their nodes (Fig. 1C).

Hybrid Confirmation Through SSR Markers

The hybrid was confirmed through SSR markers (Supplementary Fig. 1B) at molecular level and

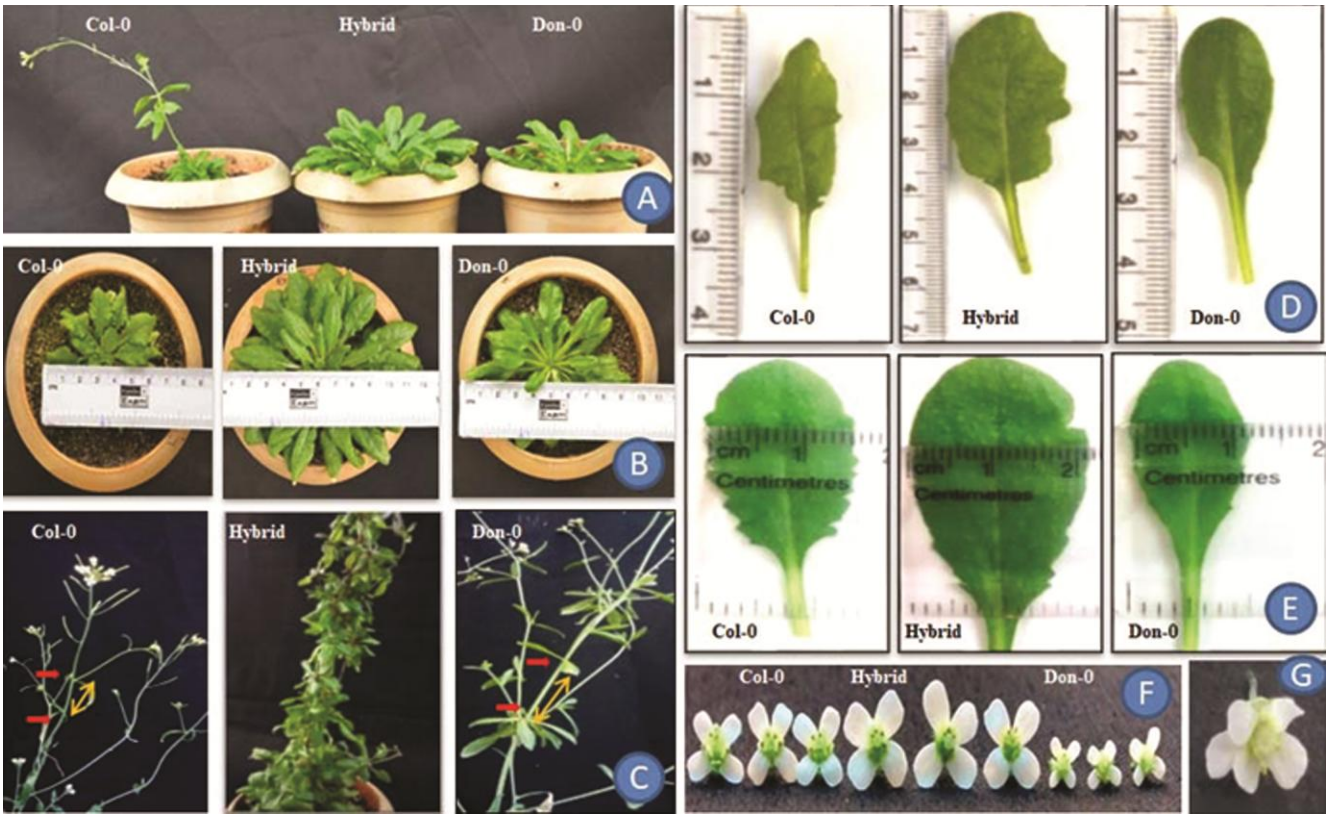

Fig. 1 — Image showing different phenotypic trait variability among parents (Col-0 and Don-0) and their hybrid. (A) Early bolting and flowering of Col-0; (B) Rosette diameter and number of rosette leaf; (C) Cauline leaf and internode traits (internode number and distance) after flowering; (D) Rosette leaf length; (E) Rosette leaf width; (F) Flower diameter; (G) flower exhibited five petals (new flower phenotype).

hybrid vigour at morphological level. A set of 10 highly polymorphic SSR selected and deployed over parents (C0l-0 and Don-0) to identify polymorphic SSRs. Out of 10, 4 were found to be polymorphic which then used to amplify 42 F_1s alongwith parents following the procedure described[30]. Out of 42, 41 F_1s were found to be true hybrid and out of which 6 were used to carry out further analysis.

Comparative Study of Hybrid With Their Parents

The true hybrids were grown along with parents under glasshouse conditions for measurement of developmental data of several morphological traits. The life cycle and their different developmental stages were shown in Figure 2A. The hybrid showed heterosis for most of the traits including root and shoot biomass (Fig. 1 and Fig. 3). For instance, number of rosette leaves and cauline leaves were high in hybrid as compared to both the parents (Fig. 1B and 1C; Fig. 4C and 4E). Hybrid exhibited late bolting and flowering as compared to both the parents (Fig. 5E). Morphology of rosette leaf shows variation that exhibited marginal leaf serration in Col-0 and smooth leaf margin in Don-0 (Fig. 1E).

Developmental Data of Col-0, Don-0 and Hybrid for Shoot and Root Related Traits

Detailed phenotypic data was scored in the gap of every seventh day for study of their growth pattern (supplementary Fig. 2) that covers the plant developmental dynamics which is following:

1. Rosette Leaf and Trichome Density Traits

Rosette Leaf Length, Width, Diameter and Number of Rosette Leaves

Rosette leaf length and width was found to be high in hybrid as compared to both the parents at 27 days and gradually increases and become constant after 104 days (Fig. 1D and 1E; Fig. 4A and 4B). Rosette leaf length of Don-0 (3.18 cm) was larger than Col-0 (2.54 cm) at 48 days and less than hybrid (4.56 cm). Maximum leaf length was found at 111 days in hybrid (6.88 cm) as compared to both the parents. The leaf

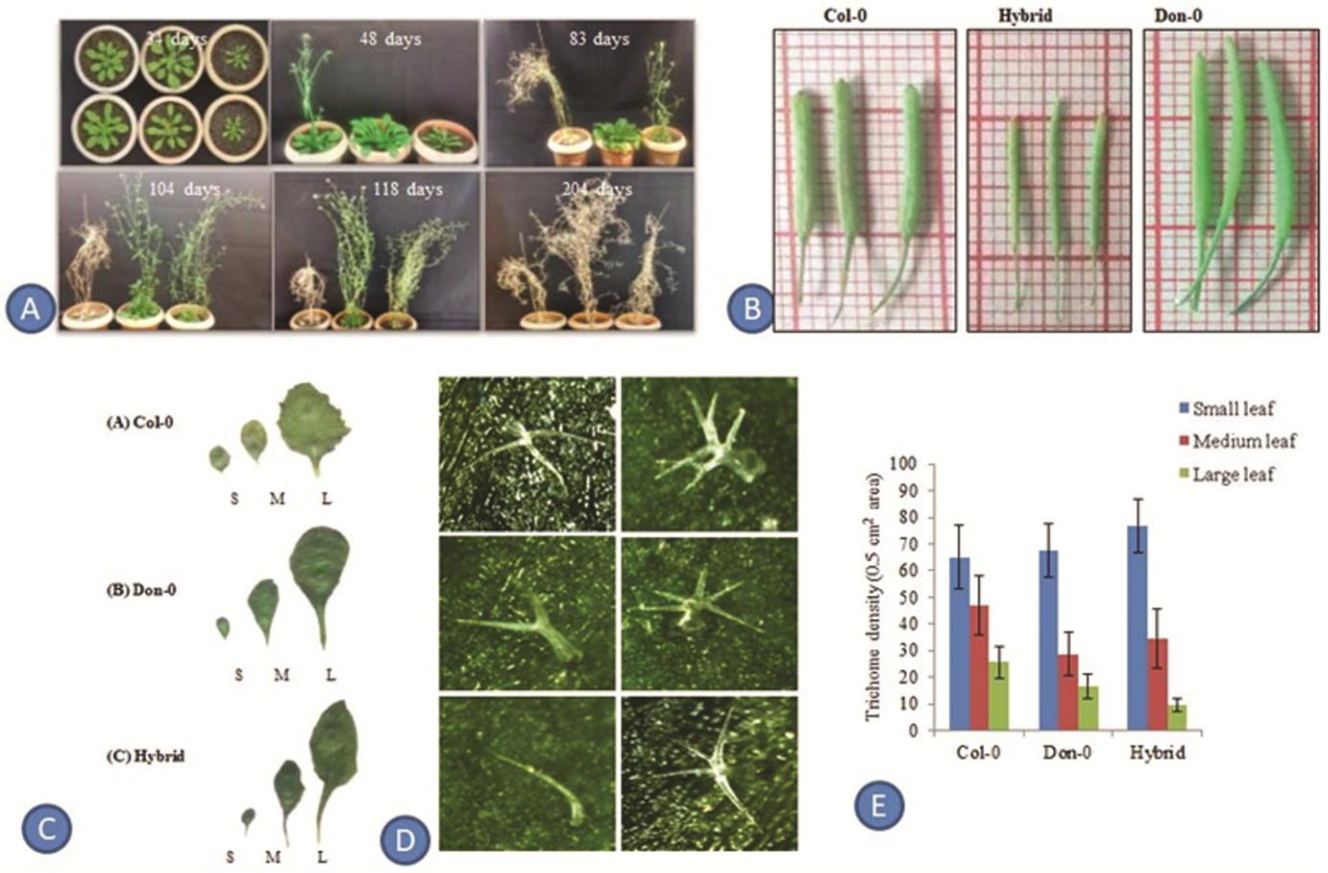

Fig. 2 — Image showing (A) Life cycle of Col-0 (left), Don-0 (right) and hybrid (middle) exhibited comparative plant height, (B) Variation for siliques length, (C) number of trichomes among Col-0, Don-0 and hybrid in small (S), medium (M) and large (L) leaf and (D) Diversity in structure and branching pattern of trichome in hybrid and (E) Bar plot showing trichome density in different size of leaf.

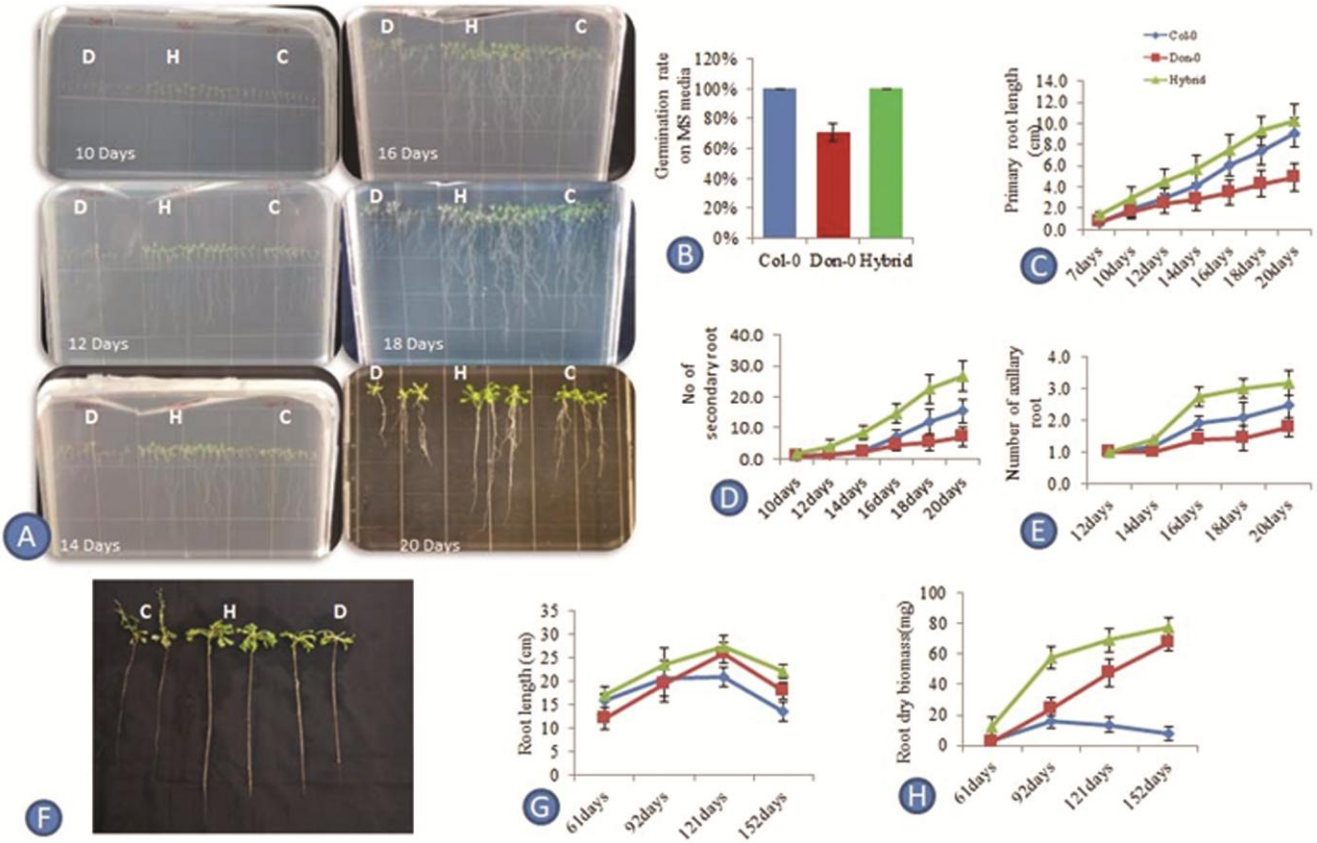

Fig. 3 — Representation of phenotypic variation for root traits. (A) Root growth dynamics on MS agar media with particular day interval (C: Col-0, D: Don-0 and H: Hybrid). Root image was captured within MS media from 10 to 18 days and outside the MS media at 20 days respectively. Graph showing (B) Germination rate; (C) Primary root length; (D) Number of secondary roots; (E) Number of axillary roots; (F) Root length on soil media. Measurement after harvesting from soil for; (G) Root length and (H) Root dry biomass.

width was high in hybrid (2.39 cm) followed by Col-0 (1.88 cm) and Don-0 (1.73 cm) at 83 days. Rosette diameter of Col-0, Don-0 and hybrid was 5.3 cm, 6.2 and 9.3 cm at 27 days respectively (Fig. 5A). Largest rosette diameter was noticed in hybrid (Fig. 1B) and reached their maximum size at 83 days (13.1 cm) which was high as compared to Col-0 (7.7 cm) and Don-0 (10.9 cm). After 83 days there is no enhancement of rosette diameter in hybrid and parents. At very initial level (27 days), less number of rosette leaves was counted in Don-0 as compared to Col-0 ecotype. Later, the number of rosette leaves was found to be high in Don-0 but less than hybrid. The number of rosette leaves was reached their higher number in Col-0 (63 leaves), Don-0 (87 leaves) and hybrid (144 leaves) at 90, 97 and 139 days, respectively (Fig. 4C).

Trichome Density

Trichome density was observed in three stages of rosette leaves, first was new leaf (small size leaf), second partial mature (medium size leaf) and third fully mature leaf (large size leaf; Fig. 2C). Small leaf showed higher number of trichomes in hybrid (77 trichomes) as compared to Col-0 (65 trichomes) and Don-0 (68 trichomes) (Fig. 2E). In case of medium leaf, trichome number was high in hybrid (34 trichomes) as compared to Don-0 (29 trichomes) but less than Col-0 (47 trichomes) (Fig. 2E). In contrast to small leaf, large leaf exhibited maximum number of trichomes in Col-0 (26 trichomes) as compared to Don-0 (17 trichomes) and hybrid (10 trichomes) (Fig. 2E). Finally, it has been observed that number of trichomes was high in small size leaf followed by medium and large size leaf for Col-0, Don-0 and hybrid in similar pattern. The pattern of trichome distributions was variable as counted among all three type of plants leaf in Col-0, Don-0 and hybrid. It means trichome number was initially high in hybrid as compared to Col-0 for small leaf and gradually decreased in large leaf. As results indicated that trichomes spreaded throughout the leaf due to

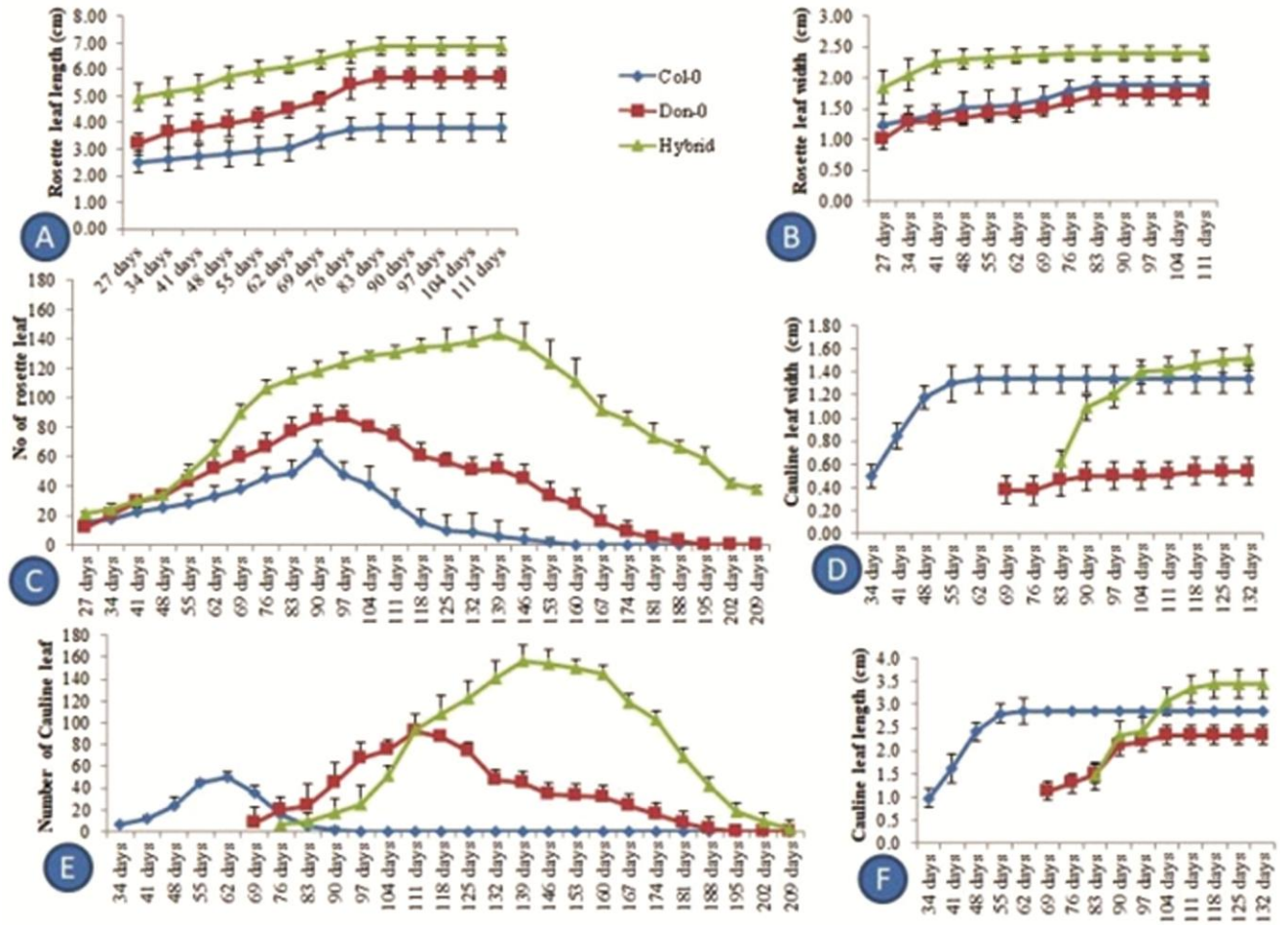

Fig. 4 — Comparative study between Col-0, Don-0 and hybrid for morphological traits. Graph showing Developmental data of leaf traits. (A) Rosette leaf length; (B) Rosette leaf width; (C) Number of Rosette leaf; (D) Cauline leaf width; (E) Number of cauline leaf; (F) Cauline leaf length.

expanded leaf area (trichome number not increased) because leaf length and width reached their maximum in large size leaf. Different type of trichome morphology was found in hybrid leaf ranged from single branch to six branch of trichome (Fig. 2D).

2 Bolting Days, Days to Flowering and Flower Diameter

The hybrid exhibited delayed bolting and flowering (average: 95.5 and 105 days) as compared to parents (Fig. 1A and 5E). Flower diameter of Col-0 (0.4 cm) was less than hybrid (0.5 cm) but higher than Don-0 (0.3 cm) as shown in Fig. 1F and 5F. In addition, 5 petals containing unique type of flower was also observed during the present study (Fig. 1G).

3. Cauline Leaf and Internode Traits

Cauline Leaf Length, Width and Number of Cauline Leaves

The cauline leaf length and width were measured with first originated leaf of first, second and third node of plant from juvenile to mature stage (Fig. 1C). The cauline leaf length and width were reached at their maximum level at 62 days, 104 days and 132 days for Col-0, Don-0 and hybrid, respectively (Fig. 4D and 4F). Don-0 represented less cauline leaf length (2.3 cm) and width (0.54 cm) as compared to Col-0 (length: 2.9 cm, width: 1.34 cm) and hybrid (length: 3.5 cm, width: 1.52 cm). The maximum number of cauline leaves was counted at 62 days (51 leaves), 111 days (93 leaves) and 139 days (156 leaves) in Col-0, Don-0 and hybrid, respectively (Fig. 4E). The hybrid showed higher number of leaves as compared to both parents (Fig. 2A and 4E). The Col-0 showed early bolting and flowering as compared to both Don-0 and hybrid so that their leaf initiation to maturation cycle accomplished very fast.

Number of Internodes and Internode Distance

The distance between two nodes has been measured as one internode distance and same for

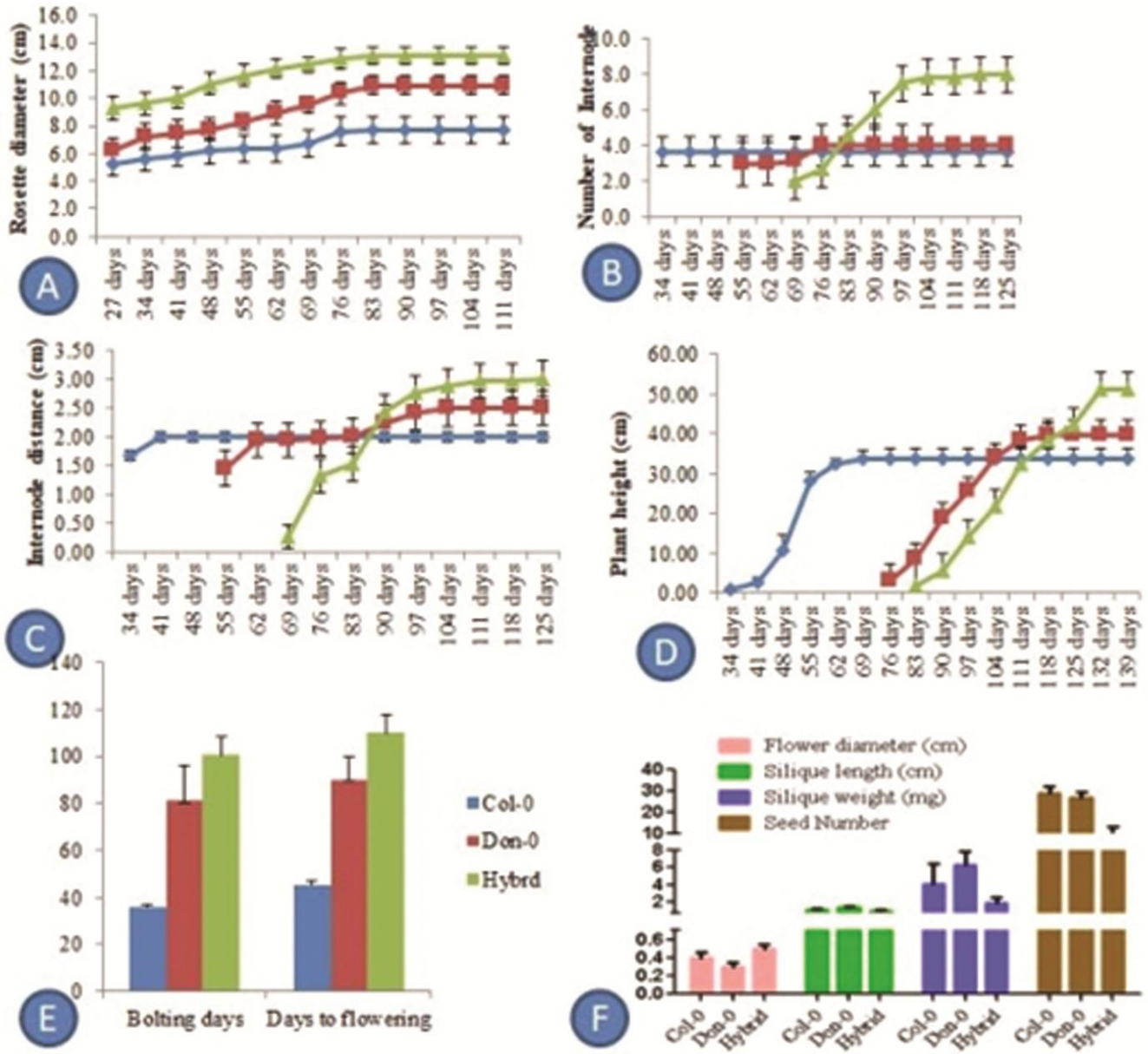

Fig. 5 — Comparative study between Col-0, Don-0 and hybrid for morphological traits. (A) Rosette diameter; (B) Number of internode; (C) Internode distance; (D) Plant height; (E) Bolting days and days to flowering. (F) Flower diameter, silique length and weight and seed number.

their number counted (one internode is counted between two nodes; Fig. 1C). Internodes number were not varied significantly between Col-0 (3.7) and Don-0 (3.8) but counted high in hybrid (8.0) as compared to both parents at 104 days (Fig. 5B). In case of internode distance, Don-0 (2.51 cm) showed higher internode distance as compared to Col-0 (2.01 cm) but less than hybrid (3.10 cm) at 125 days (Fig. 5C).

4. Plant Height and Silique Related Traits

Plant Height

The plant height was measured first in Col-0 due to its early development (fast stem initiation/growth, early bolting and flowering) as compared to Don-0 and hybrid (Fig. 2A). Subsequent to that plant height of Col-0, Don-0 and hybrid was measured which reached at their maximum height that was 33.90 cm, 39.70 cm and

51 cm at 69 days, 118 days and 132 days respectively (Fig. 5D).

Siliques Length, Weight and Seed Number

The siliques length, weight and seed number considered as yield traits and reproductive success. The silique length was high for Don-0 (1.4 cm) as measured to Col-0 (1.1 cm) and hybrid (1.0 cm) (Fig. 2B and 5F). Silique weight of hybrid (1.9 mg) was less than both parents i.e. Col-0 (4.1 mg) and Don-0 (6.3 mg). However, average seeds number was high in Don-0 (26.9) as compared to hybrid (9.4) but less than Col-0 (29.1) (Fig. 5F).

5. Root-related Traits on MS Agar and Soil Media

The hybrid and Col-0 showed 100% germination rate when grown on MS agar media while Don-0 exhibited 66-75% germination rate (Fig. 3B). Primary, secondary and axillary root length and their number was measured with one day interval (Fig. 3A).

Primary, Secondary and Axillary Root Data Analyses on MS Agar Media

After 7 days of germination, the primary root length was almost equal in both Col-0 and Don-0 that was 0.7 cm (Fig. 3A). The hybrid showed larger primary root length in all the days as compared to both the parents (Fig. 3C) and reached at maximum root length at 20 days (10.3 cm) which was also higher than Col-0 (9.1 cm) and Don-0 (4.9 cm). Similarly the number of secondary roots was similar in both parents (10 days) and high in hybrid which gradually increased with days (Fig. 3D). After 20 days (Fig. 3A), number of secondary roots was high in hybrid (26.7 cm) as compared to Col-0 (15.4 cm) and Don-0 (7.1 cm). Origination of number of axillary roots was high in hybrid which showed their maximum number (3.2) as compared to both Col-0 (2.5) and Don-0 (1.8) at 12 days. Hybrid showed high number of axillary roots followed by Col-0 and Don-0 respectively (Fig. 3E).

Root Length and Root Dry Biomass on Soil Media

The seeds of Col-0, Don-0 and hybrid were also grown on soil media under glass house conditions and plants were harvested for further analyses of root related traits (Fig. 3F). It was found that root length of Col-0 was large (16 cm) as compared to Don-0 (12 cm) but less than hybrid (17.2 cm) at 61 days. In contrast to it Don-0 gradually increased their length and showed high root length as compared to Col-0 and less than hybrid at 121 and 152 days (Fig. 3G). Similarly root dry biomass of Don-0 was less than (2.4 mg) both hybrid (12.8 mg) and Col-0 (3.6 mg) at 61 days and it was higher than Col-0 in later stages. In case of hybrid, root length and dry biomass was high as compared to both of the parents for most of the days (92,121 and 152 days, Fig. 3H).

Discussion

The traits related to biomass and flowering days are important due to their association with fitness, productivity and reproductive success of plants. The Col-0 and Don-0 is diverse at ecological level as they found in different environmental conditions and location (Col-0: United States of America and Don-0: North African, Spain). Wang *et al*[27] constructed the phylogenetic tree of 80 accessions (including Col-0 and Don-0) based on pseudogenes and concluded that Col-0 and Don-0 were diverged at molecular level. In addition, the whole genome sequencing of eight regions populations (containing 80 strains) have been performed and concluded that Don-0 (Iberian Pen./North Africa population) showed higher number of unique SNPs[28-30]. These previous reports suggested pre-existence of diversity of Col-0 and Don-0 ecotypes at ecological as well as at molecular level. The diverse parents used to develop hybrids considered to exhibit high degree of heterozygosity and transgressive segregants which helps to understand the various phenotypic, physiological and molecular mechanism of plant system. The hybrid developed in the present investigation exhibited considerable degree of hybrid vigour for most of the traits that indicates some characteristic retained from Col-0 (large flower size) and others like Don-0 (aerial rosette of cauline leaves on nodes) (Fig. 1). The heterosis may change the biological events and molecular pathways which ultimately exhibited in the form of superior phenotypic traits due to interactions of alleles and between loci[31] and/or creation of new genetic combination. Usually, heterosis is genetically intriguing phenomenon governed by (1) *Dominance effect*: suppression of undesirable or deleterious recessive alleles, (2) *Over-dominance effect*: over-expression of certain genes in the heterozygous offspring and (3) *Epistasis effects*: interactions between alleles of different loci[32]. Prior analysis was performed through hybrid development of various *A. thaliana* ecotypes includes C24 and Ler[33-34], Col-0, C24, and Nd and five hybrids from Col-0, Ws-2, Wei-0, Aa-0, C24. The developmental data of hybrids exhibited prominent phenotypic traits

from Col-0 (flower diameter, trichome density, rosette leaf width, cauline leaf length and width) and other from Don-0 (rosette leaf diameter with their leaf length, number of rosette and cauline leaves, internode distance, plant height and root biomass). However, the rosette diameter, length, width and number of rosette leaves were high in hybrid due to consumption and movement of their energy towards vegetative stage other than reproductive stage (delay flowering and reduced seed yield). As their life cycle depicts, variation in number of rosette and cauline leaves indicates that plant used their energy to originate continuous new leaves till their maturity. Higher number of leaves (leaf biomass) is basically required for proper photosynthesis, productivity and other metabolic activities of plants. Low performance of hybrid for silique length, weight and seed numbers as compared to leaf traits indicates towards the hypothesis that the hybrid probably utilized their energy for biomass enhancement or accumulation but not for yield production. Don-0 exhibited delayed flowering as compared to Col-0 which may be one of the reasons for high rosette biomass of Don-0 (Fig. 1B). Similarly, the Don-0 showed delayed flowering as compared to both Ler and Col-0 in long day photoperiod condition but changed under short day environment[35] due to sensitivity against temperature and their origin (occurrence of Don-0 in lower longitudes -6.36° condition)[36]. However, trichome density was high in small leaf as compared to large leaf (Fig. 2C and 2E) because trichome numbers were not increased with leaf size. Although, when leaf area enlarged so that trichomes spreaded throughout the leaf. The plant height and shoot traits associated to plant fitness and reproductive success (seed yield)[37]. Initially Don-0 showed less root length as compared to Col-0 on MS agar media (10-20 days) but higher length and dry biomass at maturity (141 days and 172 days) in soil media under same glass house condition (Fig. 3A, 3G and 3H).

In *A. thaliana*, root related traits have not been analyzed using Don-0 till date but there is some other reports are available in which root length[38-39] and root biomass trait[40] studied using different ecotypes. Some root related traits were investigated under physical constraints[41] drought stress, low potassium like conditions but they did not explained root heterosis in hybrid except F_1 of Col-0 x Ct-1[42]. Swiadek *et al* developed hybrid in *A. thaliana* (Alt-5 x Bod-6) and interpreted same results as found in the present investigation for flowering (delay) and seed yield (less) due to interaction or presence of parental alleles in heterozygous condition of hybrid. In the present investigation, high shoot biomass (number of rosette and cauline leaf with their length and width) and root biomass of hybrid due to over-dominace, in contrast to seed trait. Low seed yield was governed by hybrid necrosis (hybrid incompatibility) phenomenon caused by deleterious epistatic interactions and important factor for evolution[43]. It might be be due to combination of parental alleles/loci synergistically govern the phenotypic traits due to their presence in specific coding or non-coding region of genome. The combination and effect of alleles would be different in hybrid as compared to Col-0 and Don-0 due to sharing of two parental chromosomes with diverse genetic makeup. However, it is investigated that occurrence of SNP in genome could be linked with root traits, stem height, rosette and cauline leaf traits, flowering and seed traits in *Arabidopsis*[44-46]. Interestingly there is only one report in which a total of 779 candidate genes were investigated within 10-kb genomic regions of heterosis-associated SNPs detected through annotation (Yang *et al*. 2017) Although, spatiotemporal allele organization by allele-specific CRISPR live-cell imaging (SNP-CLING) technique used to validate the specificity and accuracy of heterozygous SNPs[47]. Depth study of hybrid and /or heterozygous genotypes may give the information related to contribution of superior allele in heterosis process for trait enhancement govern by positive dominance, over-dominance process[48]. In addition, contribution of active novel alleles may be explored from selected parents of *Arabidopsis* to detect the association and regulation of particular traits specifically from Don-0 due to presence of maximum number of unique SNPs in genome. Pre-existing report suggested that more active allele of Don-0 ecotype was found for flowering-RNA COLDAIR (Mendez-Vigo *et al.* 2016) which could be further utilized to explore the trait-related new candidate gene (active or superior allele) that may involved in trait specific metabolic pathways and biological processes.

Conclusion

The distinct and visible heterosis was observed for most of the phenotypic traits. The hybrid vigor and hybrid necrosis were associated with biomass related traits (rosette and cauline leaf length, width and their number, root length and biomass) and yield

traits (silique length, weight and seed number) respectively. The developmental data and heterosis analysis suggested that high shoot and root biomass of hybrid (hybrid vigour) could be due to positive association or interactions of alleles or loci in heterozygous condition. The further investigation on these traits may generate new information (unique active alleles/loci) and genetic resources for respective traits. Furthermore, study on contribution of epigenetic loci (associated with environmental effect) would also be more informative to better understand the regulation of both hybrid vigor and hybrid necrosis in plant system.

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